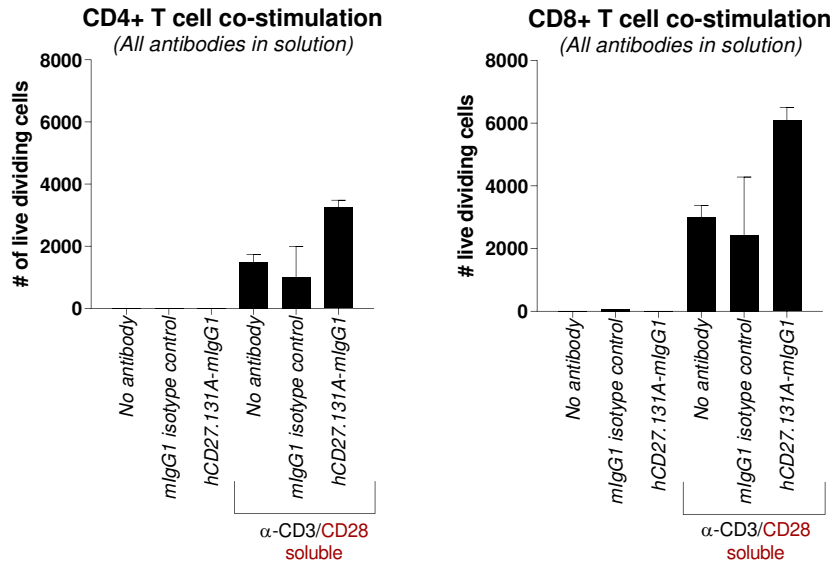
Additional File 3: In vitro co-stimulation assay data

1) Dependence of anti-CD27-mediated human T cell proliferation on TCR activation.

Human T cells were isolated from buffy coats using the Pan T-cell isolation kit (Miltenyi Biotec) according to the manufacturer's instructions, and labeled with 1 µM CFSE. Next, T cells were incubated for 3 days with or without anti-CD3 (Clone OKT-3, 0.125 µg/ml) and CD28 (Clone CLB-CD28/1, 15E8, 1.0 µg/ml), in combination with No antibody, Isotype control or hCD27.131A-mIgG1 at 10 µg/ml. All antibodies were added in solution. Next, cells were stained with PE-Cy7 Mouse Anti-Human CD4 (Clone SK3) and APC-H7 Mouse Anti-Human CD8 (Clone SK1) and analyzed by flow cytometry. Proliferating CD4+ and CD8+ T cells were quantified by determining the number of cells displaying reduced CFSE intensity. As is shown in Figure 1, in the absence of anti-CD3/anti-CD28, hCD27.131A-mIgG1 is not capable of enhancing CD4+ or CD8+ T cell proliferation. Treatment of T cells with anti-CD3/anti-CD28 results in modest induction of CD4+ and CD8+ T cell proliferation (conditions with No antibody or Isotype control), which is further enhanced by hCD27.131A-mIgG1.

Figure 1 Dependence of hCD27.131A-mediated T cell activation on anti-CD3/anti-CD28 costimulation



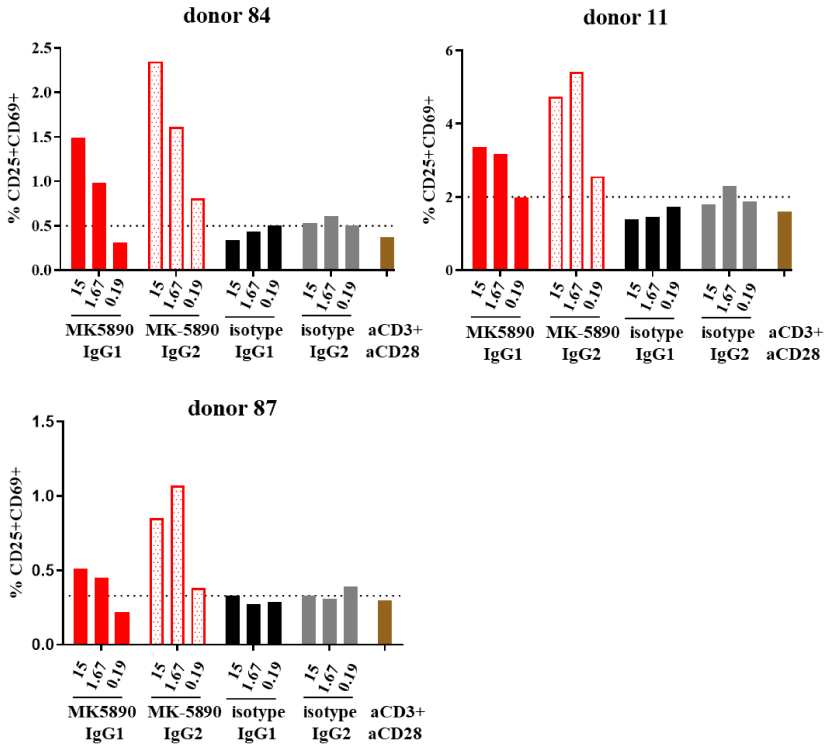
Co-stimulation of human T cell proliferation by hCD27.131A-mIgG1 in absence or presence of anti-CD3/anti-CD28. T cells were isolated, labeled with CFSE, and treated for 3 days with or without anti-CD3 (Clone OKT-3, 0.125 µg/ml) and CD28 (Clone CLB-CD28/1, 15E8, 1.0 µg/ml), in combination with No antibody, Isotype control or hCD27.131A-mIgG1 at 10 µg/ml. All antibodies were added in solution. Proliferation of CD4+ T cells (left) or CD8+ T cells (right) was analyzed by flow cytometry and plotted as the number of live, dividing cells. Error bars represent the mean of triplicate measurements with range. hCD27.131A-mIgG1 is capable of enhancing T cell proliferation only in combination with anti-CD3/anti-CD28.

2) Similar co-stimulation of CD8+ T cell activation with human IgG1 and human IgG2 isotypes of MK-5890

Enriched CD8+ cells were re-suspended to 7.5 × 10^5 cells/mL in DMEM-F12, 5% heat inactivated human serum, 50 µM 2-mercaptoethanol, 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were cultured for 3 days at 1.5 × 10^5/well in the presence of a sub-optimal dose of 0.025-0.05 µg/mL αCD3 mAb (clone OKT3, BioLegend) and 1 µg/mL αCD28 mAb (clone 15E8, Millipore) with at most 15 µg/mL soluble anti-CD27 antibody in a flat-bottom 96-well plate. Following stimulation, cells were washed with PBS and stained with a fixable viability dye (eBioscience) for 30 minutes at 4°C, washed and blocked with TruStain FcX (BioLegend). Cells were incubated with phenotyping antibodies for 30 minutes at 4°C before being washed, fixed, and acquired on the BD LSRII or Fortessa™ flow cytometer (BD Biosciences). Activation of T cells was measured using the surface markers CD25 and CD69.

As expected in this assay which has no FcγR-positive cells present, the costimulatory agonist activity of MK-5890 is retained when MK-5890 is converted to an IgG2 isotype. (Fig 2).

Figure 2 Co-stimulation of purified CD8 T cells by IgG2 isotype of MK-5890



Naïve human CD8+ T cells were activated with a suboptimal concentration of anti-CD3 mAb and co-stimulated with anti-CD28 mAb in the presence of 15, 1.67, or 0.19 µg/mL anti-CD27 or isotype control mAbs and cultured for 3 days. Next, activated CD25+CD69+ CD8+ T cells were detected by flow cytometry. Method details and Representative flow cytometry plots can be found in Additional File 1.

3) Lack of induction of IFNγ by MK-5890 hIgG2 in primary human tumor culture assays

Fresh non-small cell lung cancer (NSCLC) tumor tissues were processed into single cells suspensions by fine cutting with a scalpel, followed by a 30-minute incubation at 37°C in digestion media composed of 10 mL DMEM with 0.5 mg/mL collagenase type I, and 400 U/mL DNase I. Single cells were separated from undigested material with 70 µM strainer and washed. If cell viability was less than 30%, a Ficoll-density gradient separation was performed to enrich live cells. A total of 0.1×10^6 enriched live cells containing a mixture of various cell types from the tumor per well were cultured in 96-well round-bottom plates and stimulated with 10 ng/mL soluble anti-CD3 (BioLegend, clone OKT3) in the presence of indicated concentration of MK-5890 (hIgG1) or MK-5890 hIgG2, 10 µg/mL pembrolizumab, or 20 µg/mL IgG1 or IgG2 isotype control mAb. The supernatants were collected on day 6 and IFNγ was measured using a human IFNγ tissue culture kit (Meso Scale Discovery).

Similar to the loss of MK-5890 activity in this assay when the Fc is mutated to prevent FcγR binding (main paper Fig 2D), conversion of MK-5890 to hIgG2, an isotype with minimal FcγR binding, results in loss of activity in this assay (Fig 3 below).

Figure 3 Induction of IFNγ by MK-5890 in primary human tumor culture assay is lost when MK-5890 is converted to a hIgG2 isotype.